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Malaria chemotherapeutic studies in mice included 6 different testing systems. **1)** A primary antimalarial blood schizonticidal test (MM) where in 5 years 26,488 compounds were evaluated against ***Plasmodium berghei*** with 4,294 exhibiting antimalarial activity. **2)** A casual prophylactic test (RP) to detect compounds active against the tissue schizonts where in 5 years over 5,544 compounds were tested with 527 active. **3)** A secondary antimalarial test (Ag) were in 5 years over 1,250 compounds were evaluated in a variety of different protocols. Compounds were tested for cross resistance patterns against a battery of drug-resistant parasites. A new line moderately resistant to mefloquine was developed. Resistance to mefloquine, quinine, dapsone, and cycloguanil was retained for at least 42 months while resistance lasted only 9 months to chloroquine and pyrimethamine. Six new synergistic drug

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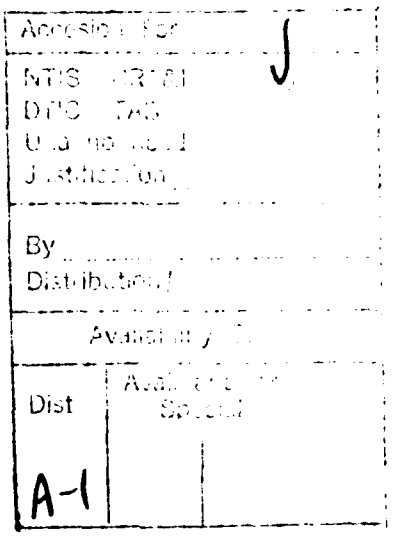
was retained for 17 months with 27 of 103 compounds while 2 of 15 were active for 60 days and 4 were inactive for 120 days duration. **4)** In a primary screen for African trypanosomes (RR) 18,241 compounds were tested with 770 exhibiting activity. **5)** In a drug-resistant African trypanosome test 150 compounds were tested against lines resistant to either melarsoprol, suramin or pentamidine. **6)** In a Chagas' disease test 300 compounds were tested with 25 exhibiting activity.



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FOREWORD

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PI Signature Celia L Cagle **Date** 6/19/91

INTRODUCTION

Malaria is the most important infections disease the world has ever known. Over 400 million people are estimated to be infected with malaria resulting in over 1.5 million deaths each year. Several reasons account for the inability attributed to controlling malaria. Foremost concerns the lack of effective drugs available to combat drug-resistant *Plasmodium falciparum*. Second the lack of a safe effective prophylactic agent against all species of malaria. Third the lack of a safe effective drug to replace primaquine (a toxic drug) which would be effective against the hypnozoite stage (to block relapses of *Plasmodium vivax*). Fourth concerns ineffective vector control and lastly the fact that no vaccine is available. Because of the serious problem of *P. falciparum* being resistant chloroquine and related 4 aminoquinolines, quinine and other drugs in different chemical classes there is an urgent need for new agents from novel classes active against drug-resistant malaria. *In vivo* animal model systems have proven reliable to assess new compounds against malarial infections. We have established a drug testing system (MM test) to identify new compounds against the asexual blood stages of malaria which has proved to be reliable and is able to identify all previously known antimalarial agents. This test is also useful in identifying the most active analogs of a compound emerging from a lead directed drug synthesis program.

New compounds active against the exoerythrocytic and hypnozoite stages of malaria are needed to replace the toxic primaquine. Samples of nine different chemical classes of compounds have exhibited activity against the liver stages of malaria, but primaquine remains the only one used today. It can not be prescribed as a prophylactic compound because of toxicity problems. It has poor therapeutic index and has been shown to cause hemolytic anemia in person with a deficiency in the enzyme glucose 6-phosphate dehydrogenase.

We have developed a test (RP test) in mice using *Plasmodium yoelii* parasites and *Anopheles stephensi* mosquitoes. This test can identify all know tissue schizonticidal agents and has identified and ranked according to activity several hundred compounds.

Active compounds emerging from the MM test system are selectively tested in a special secondary test system (Ag test). A wide variety of tests are performed in this test system. One aspect of this system is to identify cross resistance patterns of new active compounds against established antimalarial drugs.

We have lines of parasites resistant to all categories of drug resistant types and use them to find an active compound exhibiting no cross resistance with any standard antimalarial in a multiple dose (6-day test) system.

Another approach to antimalarial chemotherapy is by using combinations of synergistically active compounds such as Fansidar® (pyrimethamine plus sulfadoxine). Unfortunately this combinatorial drug regimen shares toxicity problems due to the sulfadoxine component. New synergistically active drug combinations are needed. We have a model system to identify synergistic interactions of compounds against malaria.

The rate at which drug resistance is attained to a specific compound is very important. Resistance to pyrimethamine has been achieved within two passages whereas, resistance to chloroquine takes more than 20 passages. We have developed a standardized procedure to measure the time required to induce resistance to a specific compound. This has been used for single compounds and also for multiple drug combinations.

Long lasting antimalarial agents would be beneficial to combat malaria. We have developed a test system (repository test) to identify such compounds and determine the duration of their repository activity.

According to the World Health Organization, there is no adequate information on the prevalence of human African trypanosomiasis. The best estimates report that 35 million people are exposed to the risk, and about 9,000 new cases are reported annually. African trypanosomiasis has a very high mortality rate and has considerable importance as a public health problem, especially in this age of increasing foreign travel.

No new antitrypanosome drugs have been introduced since the 1950's. Four drugs are currently available in the treatment of human trypanosomiasis caused by *Trypanosoma rhodesiense* or *Trypanosoma gambiense*. Three of these drugs, suramin, nitrofurazone and pentamidine are used in the treatment of the blood parasite, but lack efficacy in the treatment of central nervous system infections. The fourth drug, melarsoprol, is used in the treatment of central nervous system infections.

While these drugs are at times effective, they all have disadvantages. Suramin may cause renal damage, exfoliative dermatitis and has been shown to be teratogenic in rats. Nitrofurazone is toxic to the central nervous system and causes hemolytic anemia in glucose 6-phosphate deficient patients.

Pentamidine may cause fatal hypertension, hypoglycemia and diabetes. Administration of melarsoprol leads to lethal encephalopathy in 10 to 15 percent of cases.

New compounds active against the drug susceptible trypanosomes are needed. We have developed a murine model (RR test) using *T. rhodesiense* which is reliable and susceptible to the standard drugs used today. Active compounds emerging from this primary RR test system are then tested for cross resistance patterns to each of three drug-resistant lines (a melarsoprol, a suramin, and a pentamidine-resistant line).

Infections by the hemoflagellate *Trypanosoma cruzi*, the etiologic agent of Chagas' disease, present a devastating public health problem for millions of people in Central and South America. Reduviid bugs transmit *T. cruzi* to man via the insect's infected feces causing a disease characterized by an acute phase and a subsequent chronic degenerative phase. In addition to the cultural, social and economic factors that make Chagas' disease particularly difficult to manage, the problem is compounded by the protozoan's cellular invasiveness, its pleomorphic morphological and biochemical nature. No satisfactory course of drug therapy has been found that will treat all stages of the infection while remaining non-toxic to humans. Lampit is the primary drug to use, however, it is only active in the acute stage and has serious toxicity problems. We developed a murine test system to detect compounds active against the trypomastigote and amastigote stages of Chagas' disease. All standard compounds are active in this model system.

METHODS

MM TEST

ANIMALS HOSTS

The total supply of animals needed to screen candidate compounds was obtained from our breeding colony of outbred ICR/HA mice (*Mus musculus*). Test animals weighed 18-20 grams. Weight variations in any given experimental or control group were carefully limited to within 2 to 3 grams. In any given test all animals were approximately the same age.

Animals on test were housed in metal-topped plastic cages, fed a standard laboratory diet and given water *ad libitum*. Once the infected mice had been administered the drug, they were placed in a room maintained at 28.8°C(±2°C), with a relative humidity of approximately 66%.

TEST PROCEDURE

Test animals received an intraperitoneal (IP) injection of approximately $6X10^5$ parasitized erythrocytes drawn from donor mice infected 4 days earlier with *Plasmodium berghei*. The donor strain was maintained by passage every 4 days in separate groups of mice inoculated with 0.2 cc of a 1:435 dilution of heparinized heart blood.

DRUG ADMINISTRATION

Test compounds were dissolved or suspended in peanut oil before they were administered subcutaneously (SC). Compounds to be administered orally (PO) were mixed in an aqueous solution of 0.5% hydroxyethylcellulose-0.1% Tween-80 (HEC).

Treatment consisted of a single dose given SC or PO 3 days postinfection. At the time of treatment a 10-15% parasitemia had developed. Although the disease was well established, it had not yet caused sufficient debility to affect an evaluation of the test compound's toxicity.

Deaths that occurred before the sixth day, when untreated infected controls began to die, were regarded as the result of a compound's toxic effect and not as the result of action by the infecting parasite.

Each compound was initially administered in 3 graded doses, diluted 4-fold, to groups of 5 mice per dose level at 640 mg/kg or at the top dose of compound available for testing. Active compounds were subsequently tested at 6 or 9 dose levels, diluted 2-fold from the highest dose. Successive 6-level tests were performed at respectively lower doses until the lower limit of activity was reached, thus establishing a complete dose-response picture for that compound in a rodent system.

A drug that was toxic for the host at each of the 3 levels initially tested was retested at 6 dose levels diluted 2-fold from the lowest toxic dose.

DRUG ACTIVITY

Acceptance of a drug being sufficiently active for detailed studies was predicated on the margin between the maximum tolerated dose (MTD) and the minimum effective dose (MED) producing a significant effect. A MTD is defined as the highest dose up to 640 mg/kg causing no more than 1 of 5 animals to die from drug toxicity. The MED is defined as the minimum dose increasing the life span of treated animals by 100% over the life span of untreated controls.

Clearly inactive compounds were rejected after one test, borderline compounds were characterized by a dose-response curve which established the spread between the MTD and the lower limit of activity by determination of drug activity in the dose-level dilution test.

RP TEST

ANIMALS HOSTS

Male or female outbred ICR/HA Swiss mice, 6 to 7 weeks old, weighing 16 to 17 grams were used as test animals. They were maintained in groups of 5 and had free access to feed and water.

Mice used as a source of gametocytes (donor mice) were 8 weeks old and weighted 25 to 30 grams.

MOSQUITO COLONY

Anopheles stephensi were reared in an insectary maintained at 27.7°C(±2°C) and 70%(±2%) relative humidity with 14 hours of light and 10 hours of darkness. Larvae were fed a solution of 2.5% liver power once a day. Emerged adults were fed a 10% glucose solution *ad lib*.

INFECTED MICE AS SOURCE OF GAMETOCYTES

Donor mice to be used as a source of gametocytes were injected IP with a dilution of infected heart blood from mice previously infected with sporozoites of *Plasmodium yoelii*. These mice were used 2 to 3 days after inoculation with parasitized red blood cells. The gametocytes developed within 48 to 72 hours and produced a uniform infection in the mosquitoes.

INFECTION OF MOSQUITOES

Mosquitoes were placed in a room maintained at 21.1°C(±2°C) and 70%(±2%) relative humidity prior to receiving the infected blood meal. Donor mice harboring a 2 to 10% parasitemia were anesthetized with Nembutal and placed on top of the mosquito cages for 1 hour to allow the mosquitoes to feed on infected blood. A second infected blood meal was given the following day. Thereafter, the mosquitoes were maintained on a 10% glucose solution. A single normal blood meal was given 7 days after the first infected blood meal.

ISOLATION OF SPOROZOITES

Eighteen days after the first infected blood meal the mosquitoes were anesthetized with ether, vacuumed into plastic bags and kept immobilized on a cold table -5° to 0°C. The females were separated from the males and placed into a cold glass mortar. The males were discarded. After approximately 500 females were collected, one ml each of saline and heat inactivated mouse plasma were added and the suspension was macerated with a glass pestle for 3 minutes. An additional 20 ml of saline and mouse plasma (1:1) were added to the suspension and filtered through nylon monofilament screening fabric with a mesh opening of 90 microns. This step removed large tissue fragments of the mosquitoes, yet allowed the sporozoites to freely pass through into the suspension. This filtered sporozoite suspension was further diluted to obtain a concentration of approximately $2.5X10^5$ sporozoites per 0.1 ml of inoculum.

ADMINISTRATION OF TEST COMPOUNDS

Each compound was ground with a mortar and pestle and then suspended in a quantity of HEC to obtain the desired drug dose. The percent free base of each compound was not determined. Four hours prior the inoculation of sporozoites, compounds were administered either SC or PO at 3 graded doses diluted four-fold (160, 40 and 10 mg/kg). Groups of 5 mice per dose level were used. Subsequent tests used successively lower four-fold dilutions of test compound if mice were cured at 10 mg/kg until the lower limited of a compound's activity was reached.

Infected control mice (receiving sporozoites only) began to die due to malaria starting 7 days after inoculation of sporozoites. Deaths that occurred prior to 7 days in mice treated with test compounds were considered drug toxicity deaths. A drug that was toxic to the host at each of the 3 initial dose levels was retested at doses diluted four-fold from 10 mg/kg.

INOCULATION OF MICE WITH SPOROZOITES

Mice were injected IP with approximately $2.5X10^5$ sporozoites. Twenty of these mice were divided into 2 groups of 10 each. One group received no drug and served as a negative control. The other group was treated with WR 181023 (125 mg/kg) and acted as a positive control. One additional control group of 5 infected mice was treated with chloroquine (100 mg/kg).

DETERMINATION OF ANTIMALARIAL ACTIVITY

After the mice were inoculated with sporozoites they were placed in a room maintained at 28.8°C(°2C) and 66% (°2%) relative humidity. Antimalarial activity was determined by monitoring daily mortality. Mice which were alive after 30 days were considered cured. A compound was considered active if at least 2 mice survived for 30 days at any dose level. Active compounds were retested at 160, 40, 10 both SC and PO.

AG TEST

ANIMALS HOSTS

The testing was done in both female and male ICR/HA mice. All mice were obtained from our own breeding colony. Four week old mice were used for most experiments.

REGULAR 6-DAY TEST AND CROSS RESISTANCE DETERMINATIONS

When a new compound was obtained it was subjected to a battery of testing procedures, the extent of which depended on its degree of activity in suppressing murine malaria infections. The first test procedure was a 6-day suppressive test against the drug-sensitive P-line.

If the compound was active against the P-line, then a 6-day test against one or more drug-resistant lines followed. In this basic 6-day suppressive test, mice were divided into groups of 7 and inoculated with parasites IP. Drugs were administered twice a day, usually PO, in a volume of 10 ml/kg on the third, fourth and fifth days after inoculation of parasites. All drugs were mixed in aqueous HEC and ultrasonicated when necessary. Drug doses were prepared using 100% of the free base of each drug. One group of 10 infected mice received the vehicle alone and served as a negative control. The blood films and final group weights were taken on the sixth day after inoculation of parasites. Microscope examination of Giemsa-stained blood smears was made to determine the percentage of cells parasitized, percent suppression of parasitemias, and significance values for the suppression of parasitemias. Significance values were based on a calculation of the percent suppression of parasitemia which was determined by comparing the parasitemia of each treated mouse with the means parasitemia of the negative controls. Drugs tolerance was reflected by the percent weight change and the proportion of mice that survived treatment. Toxicity was attributed to drug action when a 14% or greater weight change occurred or when one or more mice died before the blood smears were taken.

Each new drug was first tested against the drug-sensitive P-line usually via both PO and SC routes of administration. The drug dosages for the first test were normally 64, 16, 4 and 1 mg/kg/day for 3 days. If more than 90% suppression of the parasitemia (SD_{90}) was obtained with the lower dose of 1 mg/kg/day, then testing at lower doses was performed. Chloroquine was tested as a reference

against the P-line at levels of 2, 3 and 4 mg/kg/day. A quinine index (Q) was calculated by comparing the SD_{90} values obtained from the chloroquine dose-response curve and the SD_{90} value of the new compound:

$$Q = \frac{SD_{90} \text{ of chloroquine } 30^{\cdot}}{SD_{90} \text{ of new compound}}$$

$^{\cdot}$ = This is the quinine index for chloroquine.

Compounds that suppressed the P-line parasitemia by at least 90% with 64 mg/kg or less were subjected to testing against 1 or more of the drug-resistant lines listed below;

P. berghei KBG-173

mefloquine resistant	A-line
chloroquine resistant	C-line
pyrimethamine resistant	M-line
quinine resistant	Q-line
dapsone resistant	S-line
cycloguanil resistant	T-line

Doses required for a given degree of effect, such as 90% suppression or SD_{90}'s were estimated graphically from plots made on log-probit paper. The ratio of the SD_{90}'s was used to delineate the degree of cross resistance.

SYNERGISTIC TESTS

Mice were infected IP with $5X10^4$ parasitized erythrocytes of a drug-sensitive line. The drugs were mixed separately then administered either alone or as a mixture PO twice a day on days 3, 4 and 5 after the mice had been infected. The effects were determined from blood smears made 1 day after completion of treatment. The dose suppressing 90% of the parasites (SD_{90}) for 1 drug alone and of the mixture were estimated by plotting parasitemia suppressions on probit-log scale graphs. The analyses

for synergism were based upon partitioning of the SD_{90} value of each combination in terms of its components. These components were then compared with the respective SD_{90} values of the corresponding drug alone. If the joint effects were simply additive, each component of a mixture SD_{90} would be expected to be 0.5. If all values were lower than 0.5 the data would indicated synergism. Conversely, if all values were greater than 0.5 the data would indicated antagonism.

INDUCTION OF DRUG RESISTANCE

The drug-sensitive line of *P. berghei* was used to start the induction of resistance. Each compound was first administered at the same 7 dose levels (2, 1, 0.85, 0.5, 0.38, 0.25, 0.125 mg/kg/day) for 3 consecutive days b.i.d. commencing on D+3 after infection with $5X10^4$ parasitized erythrocytes. Blood films were made on D+7 and the mouse at the highest dosage level with a parasitemia of 1-5% was used as a donor mouse. This procedure was repeated weekly using the dose level passed to be the second lowest dose (X) and then the drug was increased for the next pass according the following increments.

8X
4X
3X
2X
1.5X
0.5X
0 - No drug

By using this schedule a standardized procedure was used to assess the speed that resistance to each compound was attained.

MEFLOQUINE

Starting with the drug-sensitive P-line of *P. berghei* mefloquine was given to groups of mice at the standardized increments until resistance was attained.

REPOSITORY TEST

On day zero, mice are given a single SC or PO dose of the compound. Negative controls receive injections of the vehicle alone and routinely die six to eight days after challenge with parasites.

On days +3, +10, and +17, treated and control subgroups are challenged in parallel with approximately $5.0X10^5$ parasitized erythrocytes obtained from *P. berghei* infected donor mice. Mice are challenged only once.

Selected compounds that have displayed activity when administered 17 days prior to challenge are further tested using a similar procedure with challenges on days +30, +60, and +90 or on day +120.

Mortality over a four week period is used as an index of compound repository activity. Negative controls all die six to nine days after the challenge. Deaths occurring before the 6th day postinfection are attributed to compound toxicity. Treated animals alive four weeks after challenge with *P. berghei* are considered cured.

RR TEST

ANIMAL HOSTS

ICR/HA outbred mice were used for this test. In this screening procedure mice weighed 25 to 28 grams with weight variation in any given experimental or control group carefully limited to 3 grams. Male and female mice approximately the same age were used.

Animals were housed in metal-topped plastic cages, fed a standard laboratory diet and given water *ad libitum*. After drug treatment mice were kept in a room maintained at a temperature of 28.8°C(±2°C) with a relative humidity of 66% (±2%).

INOCULATION OF PARASITES

Test animals received and IP injection of 0.2 cc of a $1.5X10^4$ dilution of heparinized heart blood drawn from a donor mouse infected 3 days earlier (approximately $1.3X10^4$-$1.7X10^4$ trypomastigotes.

The donor line was maintained by 3-day blood passes; each animal received 0.1 cc of a $1:1.5X10^4$ dilution of heparinized heart blood drawn from a mouse harboring a 3-day infection.

One group of infected, untreated mice was included as a negative control, to check both the infectivity of the *T. rhodesiense* (CT-Wellcome strain) and the susceptibility of the murine host. In

order to determine the effect a drug exerted on a trypanosome infection, 2 parameters were measured; **1)** the increase in mouse survival time and **2)** drug curative action. For comparative purposes, 2 standard antitrypanosomal compounds, stilbamidine isethionate and 2-hydroxystilbamine isethionate, were administered SC at one dose (26.5 mg/kg) to separate groups of 10 mice each. The same positive controls were administered PO at 53 mg/kg when compounds were tested orally. These 2 diamidines served as positive controls, producing definite increase in survival time and curative effects.

DRUG ADMINISTRATION

Test compounds were dissolved or suspended in peanut oil before they were administered SC. Compounds to be administered PO were mixed in an aqueous solution of HEC.

Treatment consisted of a single dose, given SC or PO, 2 to 3 hours after the injection of parasites. Deaths that occurred before the 4th day, when untreated infected controls began to die, were regarded as a result of toxic action by the drug, not the lethal effects of the parasites.

Each compound was initially administered in 3 graded doses diluted 4-fold to groups of 5 mice per dose level. The top dose was either 424,, 212, or 106 mg/kg, depending on the amount of compound available for testing. Active compounds were subsequently tested at 6 dose levels, diluted 2-fold from the highest dose. If necessary, successive 6-level tests were performed at respectively lower doses until the lower limit of activity was reached.

A drug that was toxic for the host at each of the 3 levels initially tested was retested at 6 dose levels diluted 2-fold from the lowest toxic dose.

DRUG ACTIVITY

Acceptance of a drug as being sufficiently active for detailed studies was predicated on the margin between the maximum tolerated dose (MTD) and the minimum effective dose (MED) producing a significant effect. A MTD is defined as the highest dose up to 424 mg/kg causing no more than 1 of 5 animals to die from drug activity. The MED is defined as the minimum dose increasing the life span of treated animals by 100% over the live span of untreated infected controls.

Clearly inactive compounds were rejected after 1 test and border-line compounds after 2 tests. Active compounds were characterized by dose-response curves, which established the spread between the MTD and the lower limit of activity by a determination of drug activity in the dose-level dilution tests. Treated animals alive at the end of 30 days were considered cured.

DRUG-RESISTANT AFRICAN TRYPANOSOME TEST

ANIMALS HOST

SAME AS REGULAR RR TEST

INOCULATION OF PARASITES

Giemsa-stained blood smears from donor mice infected 3 days earlier with *T. rhodesiense* trypomastigotes were microscopically examined to determine parasitemias (i.e., number of trypomastigotes in a field of 100 erythrocytes). One set of test animals was infected with the drug-sensitive line of parasites by receiving an IP injection of 0.2 cc of a $1:1.5X10^4$ dilution of heparinized heart blood drawn from a donor mouse harboring a parasitemia of 30-35% (approx. $1.3X10^4$ - $1.7X10^4$ trypomastigotes). Other sets of mice were similarly infected with each drug-resistant line to be tested. Blood dilutions were made such that all mice infected with the resistant lines received approximately the same number of trypomastigotes as mice infected with the drug-sensitive line.

Groups of 10 mice per group infected with the drug-sensitive line and with each drug-resistant line but receiving no drug served as negative controls.

DRUG ADMINISTRATION

Test compounds were dissolved or suspended in either peanut oil for SC administration or HEC for PO administration. Compounds were given 1 hour following challenge with trypomastigotes.

Compounds were diluted 2 or 4-fold from a level that had been projected to be fully curative. Five mice were used for each dose level.

CROSS RESISTANCE DETERMINATION

Each compound was tested against the drug-sensitive line and the 3 drug-resistant lines (melarsoprol, suramin or pentamidine resistant). Mice surviving 30 days postinfection were considered cured. The degree of cross resistance (fold resistant) was obtained by the following calculation.

$$\text{Cross-resistance (Fold resistant)} = \frac{CD_{50}\ \text{Drug-resistant line}}{CD_{50}\ \text{Drug-sensitive line}}$$

CD_{50} is the lowest mg/kg level of a compound curing at least 3 of 5 mice.

CHAGAS' TEST

ANIMALS HOST

Male ICR/HA Swiss randomly bred mice 6 to 7 weeks of age weighing 18-22 grams were used.

TEST PROCEDURE

On day zero mice were inoculated IP with approximately $1.3X10^5$ trypomastigotes in blood drawn from donor mice infected 1 week previously with *T. cruzi* trypomastigotes (Y-strain). Within 30 minutes following challenge, mice were given a single SC injection of the test compound mixed in peanut oil. Each compound was initially tested at 3 dose levels, usually 640, 160, and 40 mg/kg. The end point in activity of each active compound was determined. Mortality was recorded daily for a period of 40 days after the challenge with parasites. Blood smears were taken on day 40 to detect any trypomastigoes.

Infected negative controls received an injection of the vehicle alone. This groups consistently died within 9-15 days after the IP inoculation of parasites. A positive control drug was included in each experiment. The nitrofuran, Lampit, known to have

limited therapeutic value in treating patient with Chagas' disease, was used as a positive control.

A classification system was used to assess the relative activity of prospective compounds by comparing the life span of treated animals to the longevity of negative controls. Schizotrypanocidal activity was divided into 3 categories; positive, minimal and negative. A positive compound was one producing at least 50% increase in life span of mice over that of controls. A minimal compound produced a 20 to 49.9% increase in longevity, and a compound producing less than a 20% increase in life span was considered negative.

Active compounds prevented or delayed acute mortality. The test system as designed could not assure that mice living past the 40 day observation period were cured; if complete elimination of the parasite was not attained during the acute stage of infection and the animal survived, a chronic stage followed.

RESULTS AND DISCUSSION

MM TEST

In this primary mouse malaria test we tested 26,488 compounds for asexual erythrocytic activity. At least 4,200 of these compounds exhibited antimalarial activity. Therefore, approximately 16% of the compounds evaluated had at least suppressive activity with far fewer producing curative activity. The identity of most of these active compounds is considered discreet so I can not report on their chemical structures.

RP TEST

For this sporozoite induced causal prophylactic test over 5,544 compounds were evaluated with approximately 527 exhibiting activity. The majority of these compounds were discreet. There were some false positive results obtained for compounds having no tissue schizonticidal activity but exhibiting residual blood schizonticidal activity. These false positive compounds were not sorted out from the true causal prophylactic ones.

AG TEST

Over 200 compounds were tested both orally and subcutaneously against the drug-sensitive P-line of *P. berghei*. More than 250 compounds were tested against one or more of the 6 drug-resistant lines of malaria.

Drug pressure was removed from each of the 6 drug-resistant lines and their retention of resistance was followed. The lines resistant to either mefloquine, quinine, dapsone or cycloguanil retained their resistance for 42 months at which time the lines were frozen in liquid nitrogen to be followed for longer time intervals away from drug pressure later. The chloroquine resistant and the pyrimethamine resistant lines retained their resistance for 9 months, however when checked at 12 months the resistance was lost.

There were 8 tests performed to detect synergistic suppressive activity against drug-sensitive parasites.

COMPOUNDS	SYNERGISTIC
WR 222448 + Sulfadiazine	Yes
WR 225329 + Sulfadiazine	Yes
WR 226337 + Sulfadiazine	Yes
WR 169626 + Pyrimethamine	Yes
WR 233600 + Pyrimethamine	Yes
WR 233602 + Pyrimethamine	Yes
WR 225329 + Pyrimethamine	No
WR 225329 + Trimethoprim	No

A line moderately resistant to mefloquine was developed in 4 passes. It was approximately 4-fold resistant to mefloquine but was not stable when drug pressure was removed.

In the repository test system 103 compounds tested for activity for 17 days duration with 27 exhibiting activity. Fifteen compounds were tested for 90 days duration with showing 2 activity. Four compounds were tested for 120 days duration with no positive results.

RR TEST

In the primary drug test system for African trypanosomes (RR test) 18,241 compounds were evaluated with 779 exhibiting antitrypomastigote activity.

DRUG-RESISTANT AFRICAN TRYPANOSOME TEST

In the drug-resistant African trypanosome test system 150 compounds were tested against the 3 lines resistant to either melarsoprol, suramin, or pentamidine.

CHAGAS' TEST

In the Chagas' disease test system 300 compounds were evaluated for activity with 25 exhibiting antitrypanosome activity.

CONCLUSIONS

The primary murine malaria test (MM) continues to identify new compounds and the best analogs from lead directed synthesis program. About 16% of the total compounds evaluated were active (4,294 of 26,488).

The prophylactic antimalarial test (RP) identified 527 compounds as active out of over 5,544 tested. If a compound had prolonged blood schizonticidal activity and no tissue schizonticidal activity it could be positive in this test. These false positive results were not separated from the true casual prophylactic compounds. This was a reliable test which identified many new leads to replace primaquine.

The secondary antimalarial test system (Ag) continues to evaluate selected active compounds in a variety of different test types. The ideal new antimalarial agent should be active orally so the initial secondary testing compared oral to subcutaneous administration. Over 200 compounds were tested in this system and those exhibiting good oral activity were tested against a battery of drug-resistant parasites. Very few compounds have emerged with no observable cross resistance to the standard antimalarials. Drug resistance to mefloquine, quinine, dapsone, and cycloguanil was not lost when parasites were passed without drug pressure for 42 months. Chloroquine-resistant and pyrimethamine-resistant lines retained their resistance for 9 months but when checked at 12 months resistance was lost. These studies imply resistance is very stable to certain drugs and less stable to others. Synergistic activity was obtained with sulfadiazine and WR 228448, WR 225329 and WR 226337. Pyrimethamine acted synergistically with WR 169,626, WR 233600, WR 233602. Synergistic drug combinations are urgently needed to combat chloroquine resistant human malaria. In the repository test system 27 of 103 compounds retained activity for 17 days while 2 of 15 were active for 90 days and 4 compounds tested for 120 days were not active.

In the primary African trypanosome test (RR) 779 compounds out of 18,241 were active against the trypomastigote stage. This test was very reliable and identified several new active classes of compounds.

The drug-resistant test system for the African trypanosomes evaluated 150 compounds for cross resistance against lines resistant to either melarsoprol, suramin, or pentamidine. Several compounds emerged showing no cross resistance with these 3 standard agents.

The test to detect compounds active against Chagas' disease identified 25 as active out of 300 tested. This test was reliable and identified several new classes of active compounds.

With malaria on the increase worldwide today new compounds need to be identified to combat this major disease.